

Listing of Claims

1-4. (Canceled)

5. (Currently amended) An isolated nucleic acid molecule encoding a ~~substantially purified~~ RFX4_v3 polypeptide, wherein the polypeptide comprises:

- a) an amino acid sequence at least ~~70~~99% identical to ~~an amino acid sequence set forth as~~ SEQ ID NO: 8;
- b) ~~a conservative variant of the~~ an amino acid sequence at least 95% identical to set forth as SEQ ID NO: 8, wherein fourteen consecutive amino acids within the N-terminal sequence of the polypeptide are identical to residues 1-14 of SEQ ID NO: 8; or
- c) the amino acid sequence set forth as SEQ ID NO: 8,

wherein the polypeptide in a), b), or c) has RFX4_v3 activity, ~~and fourteen consecutive amino acids within the N-terminus of the polypeptide are at least 90% identical to residues 1-14 of SEQ ID NO: 8.~~

6. (Currently amended) The nucleic acid molecule of claim 5, ~~wherein the nucleic acid molecule comprises~~ comprising:

a nucleic acid sequence at least ~~70~~95% identical to ~~the nucleic acid sequence set forth as~~ SEQ ID NO: 37.

7. (Withdrawn and currently amended) The nucleic acid molecule of claim ~~[[6]]~~18, wherein the nucleic acid sequence is at least ~~90~~95% identical to SEQ ID NO: 37, SEQ ID NO: 38 or SEQ ID NO: 39.

8. (Currently amended) The nucleic acid molecule of claim 6, wherein the nucleic acid sequence is at least ~~90~~99% identical to SEQ ID NO: 37.

9. (Currently amended) The nucleic acid ~~sequence~~ molecule of claim 5, ~~wherein the nucleic acid sequence is~~ operably linked to a heterologous promoter.

10. (Currently amended) The nucleic acid ~~sequence~~molecule of claim [[5]]9, wherein the heterologous promoter comprises SEQ ID NO: 11 or SEQ ID NO: 12.

11. (Currently amended) A vector comprising the nucleic acid molecule of claim 5.

12. (Previously presented) An *in vitro* host cell transformed with the vector of claim 11.

13. (Previously presented) The *in vitro* host cell of claim 12, wherein the host cell is a plant cell, an animal cell, or a prokaryotic cell.

14. (Withdrawn and currently amended) A composition comprising the ~~polypeptide~~
nucleic acid molecule of claim [[2]]5.

15. (Currently amended) An isolated nucleic acid molecule that hybridizes under conditions of ~~low~~high stringency to a polynucleotide consisting of nucleotides 1-42 of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39, wherein the isolated nucleic acid molecule comprises at least 15 contiguous nucleotides of nucleotides 1-42 of SEQ ID NO:37, SEQ ID NO: 38, or SEQ ID NO: 39, and wherein the isolated nucleic acid molecule encodes a RFX4_v3 polypeptide.

16-17. (Canceled)

18. (Currently amended) ~~The~~An isolated nucleic acid of claim 17 molecule comprising a nucleic acid sequence encoding, wherein the a RFX4_v3 polypeptide comprises comprising
SEQ ID NO: 6, SEQ ID NO: 8, or SEQ ID NO: 10.

19. (Currently amended) A vector comprising the nucleic acid molecule of claim 15.

20. (Previously presented) An *in vitro* host cell transformed with the vector of claim 19.

21. (Previously presented) The *in vitro* host cell of claim 20, wherein the host cell is a plant cell, an animal cell, or a prokaryotic cell.

22-24. (Canceled)

25. (Currently amended) A method for producing a variant RFX4_v3 polypeptide, wherein the method comprises:

mutagenizing a wild-type nucleic acid sequence as set forth in SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39;

expressing the mutagenized nucleic acid sequence; and

screening the variant for a RFX4_v3 activity to identify the variant of the RFX4_v3 polypeptide, wherein the RFX4_v3 polypeptide comprises:

a) an amino acid sequence at least ~~70~~99% identical to ~~an amino acid sequence set forth as~~ SEQ ID NO: 8;

b) ~~a conservative variant of the~~an amino acid sequence at least 95% identical to set forth as SEQ ID NO:8, wherein fourteen consecutive amino acids within the N-terminal sequence of the polypeptide are identical to residues 1-14 of SEQ ID NO: 8; or

c) the amino acid sequence set forth as SEQ ID NO: 8, wherein the polypeptide in a), b), or c) has RFX4_v3 activity, ~~and the N-terminus of the polypeptide is at least 90% identical to residues 1-14 of SEQ ID NO: 8.~~

26. (Previously presented) A composition comprising a nucleic acid molecule that inhibits the binding of the polynucleotide of claim 15 to its complementary sequence.

27. (Currently amended) The isolated nucleic acid molecule of claim 15, wherein the isolated nucleic acid molecule hybridizes under conditions of high ~~stringent conditions~~stringency to the polynucleotide ~~comprising~~consisting of nucleotides 1-42 of SEQ ID NO: 37.

28. (Withdrawn and currently amended) A method for detecting a nucleic acid molecule in a biological sample, comprising:

hybridizing a polynucleotide to the nucleic acid molecule of claim 5 to produce a hybridization complex, wherein the polynucleotide hybridizes under conditions of high stringency to nucleotides 1-42 of SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39;

detecting the hybridization complex, wherein the presence of the hybridization complex indicates the presence of the nucleic acid molecule encoding RFX4_v3 in the biological sample, thereby detecting the nucleic acid molecule in the biological sample.

29. (Withdrawn) The method of claim 28, wherein the polynucleotide hybridizes to SEQ ID NO: 37.

30. (Withdrawn) The method of claim 28, further comprising amplifying the nucleic acid molecule prior to hybridizing with the polynucleotide.

31. (Withdrawn) A method of identifying a subject at risk of developing RFX4_v3 linked hydrocephalus, comprising detecting in the subject a mutation in the nucleic acid molecule of claim 5, wherein the mutation in the nucleic acid molecule alters the RFX4_v3 polypeptide, thereby identifying a subject at risk of developing RFX4_v3 linked hydrocephalus.

32. (Canceled)

33. (Withdrawn) The method of claim 31, wherein detecting the mutation in the RFX4_v3 nucleic acid molecule comprises performing a hybridization analysis with a nucleic acid probe that detects the mutation in the RFX4_v3 nucleic acid molecule.

34. (Withdrawn) The method of claim 31, wherein detecting the mutation comprises identifying an individual carrying a mutated RFX4_v3 allele, wherein the method comprises: providing a nucleic acid from the subject, wherein the nucleic acid comprises a RFX4_v3 allele; and

detecting a mutation in the nucleic acid that results in phenotypic expression of congenital hydrocephalus.

35. (Withdrawn) The method of claim 34, wherein the mutation is in the RFX4_v3 allele.

36. (Withdrawn) The method of claim 31, wherein the method comprises detecting the mutation in the RFX4_v3 polypeptide.

37. (Withdrawn) The method of claim 36, wherein the method comprises detecting an abnormality in expression of the RFX4_v3 polypeptide.

38. (Withdrawn) The method of claim 37, wherein the method detects a reduced expression of the RFX4_v3 polypeptide.

39. (Withdrawn) The method of claim 36, wherein the method comprises providing a polypeptide from the subject, and detecting a mutation in the sequence encoding the polypeptide, wherein the polypeptide comprises the RFX4_v3 polypeptide and wherein the mutation results in phenotypic expression of congenital hydrocephalus.

40. (Withdrawn) The method of claim 31, comprising obtaining a biological sample from the subject, and detecting in the biological sample the mutation in the RFX4_v3 polypeptide or in the RFX4_v3 nucleotide sequence.

41. (Withdrawn) The method of claim 40, wherein the biological sample comprises blood, amniotic fluid, plasma, or cerebral spinal fluid.

42. (Canceled)

43. (Withdrawn) The method of claim 38, wherein detecting the reduced expression of the RFX4_v3 polypeptide comprises using RFX4_v1 specific antibodies.

44. (Withdrawn) A kit for determining if a subject is a carrier of a mutated RFX4_v3 gene, wherein the kit comprises:

a reagent that specifically detects a mutation in a RFX4_v3 allele, and

instructions for determining whether the subject is at increased risk of expressing congenital hydrocephalus if the reagent specifically detects the mutation.

45. (Withdrawn and currently amended) The kit of claim 44, wherein the reagent comprises a nucleic acid probe that specifically hybridizes under ~~stringent~~ conditions of high stringency to a nucleic acid sequence of SEQ ID NO: 37, SEQ ID NO: 38 or SEQ ID NO: 39.

46. (Withdrawn) The kit of claim 44, wherein the reagent comprises an antibody that specifically binds the protein expressed by the RFX4_v3 allele.

47-48. (Canceled)

49. (Withdrawn and currently amended) An antibody that specifically binds the polypeptide encoded by the isolated nucleic acid of claim [[2]]5.

50. (Withdrawn) A method for generating a non-human transgenic animal with a knockout for the RFX4_v3 gene, wherein the method comprises disrupting an RFX4_v3 transcript, the disruption being sufficient to produce hydrocephalus in the transgenic animal.

51. (Withdrawn) The method of claim 50, wherein the non-human transgenic animal is a mouse.

52. (Withdrawn) The method of claim 50, wherein disrupting a RFX4_v3 transcript comprises:

deleting or substituting any portion of the RFX4_v3 transcript,
inserting an exogenous gene into the RFX4_v3 transcript, or
any combination thereof.

53. (Withdrawn) The method of claim 50, wherein disrupting the RFX4_v3 transcript comprises crossing one non-human transgenic animal with a second non-human transgenic animal.

54-58. (Canceled)

59. (Withdrawn and currently amended) A pharmaceutical composition, comprising:

- a) a therapeutically effective amount of the polypeptide encoded by the isolated nucleic acid molecule of claim 5, ~~a the isolated nucleic acid molecule sequence encoding the polypeptide of claim 5~~, or a therapeutically effective variant or portion thereof; and
- b) a pharmaceutically acceptable carrier.

60. (Canceled)

61. (Withdrawn and currently amended) A method of treating congenital hydrocephalus in a subject, comprising administering to the subject a therapeutically effective amount of an ~~agent~~ RFX4_v3 polypeptide to the subject, wherein the RFX_v3 polypeptide is encoded by the nucleic acid molecule of claim 5.

62. (Canceled)

63. (Withdrawn) The method of claim 61, wherein the method comprises increasing expression of RFX4_v3 polypeptide in the subject.

64. (Withdrawn) The method of claim 63, wherein the method comprises introducing into the subject a vector that expresses the RFX4_v3 polypeptide in the subject.

65. (Currently amended) The isolated nucleic acid molecule of claim 5, wherein the polypeptide comprises an amino acid sequence at least ~~80~~99% identical to an amino acid sequence set forth as SEQ ID NO: 8.

66-67. (Canceled)

68. (Currently amended) The isolated nucleic acid molecule of claim 5, wherein the RFX4_v3 activity ~~comprises inhibiting~~ polypeptide inhibits the phenotypic expression of congenital hydrocephalus.

69. (Currently amended) The isolated nucleic acid molecule of claim 5, wherein the ~~activity is the ability to~~ polypeptide encoded by the nucleic acid molecule is bound by a ~~bind to~~ RFX4_v3 specific ~~antibodies~~ antibody.

70-71. (Canceled)

72. (Currently amended) ~~An~~ The isolated nucleic acid molecule of claim 15, that ~~hybridizes under conditions of low stringency to a polynucleotide comprising~~ wherein the ~~isolated nucleic acid molecule comprises~~ at least 20 contiguous nucleotides in the region ~~between nucleotides 1 and 42 of a nucleic acid sequence selected from the group consisting of~~ nucleotides 1-42 of SEQ ID NO: 37, SEQ ID NO: 38, and or SEQ ID NO: 39.

73. (Canceled)

74. (New) An isolated nucleic acid molecule encoding a RFX4_v3 polypeptide having RFX4_v3 activity, wherein the polypeptide comprises SEQ ID NO: 8.

75. (New) An isolated nucleic acid molecule encoding a RFX4_v3 polypeptide having RFX4_v3 activity, wherein the isolated nucleic acid molecule comprises SEQ ID NO: 37.

76. (New) The isolated nucleic acid molecule of claim 5, wherein the isolated nucleic acid molecule encodes a RFX4_v3 polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO: 8, wherein fourteen consecutive amino acids within the N-terminal sequence of the polypeptide are identical to residues 1-14 of SEQ ID NO: 8.

77. (New) The isolated nucleic acid molecule of claim 5, wherein the isolated nucleic acid molecule encodes a RFX4_v3 polypeptide comprising SEQ ID NO: 8.